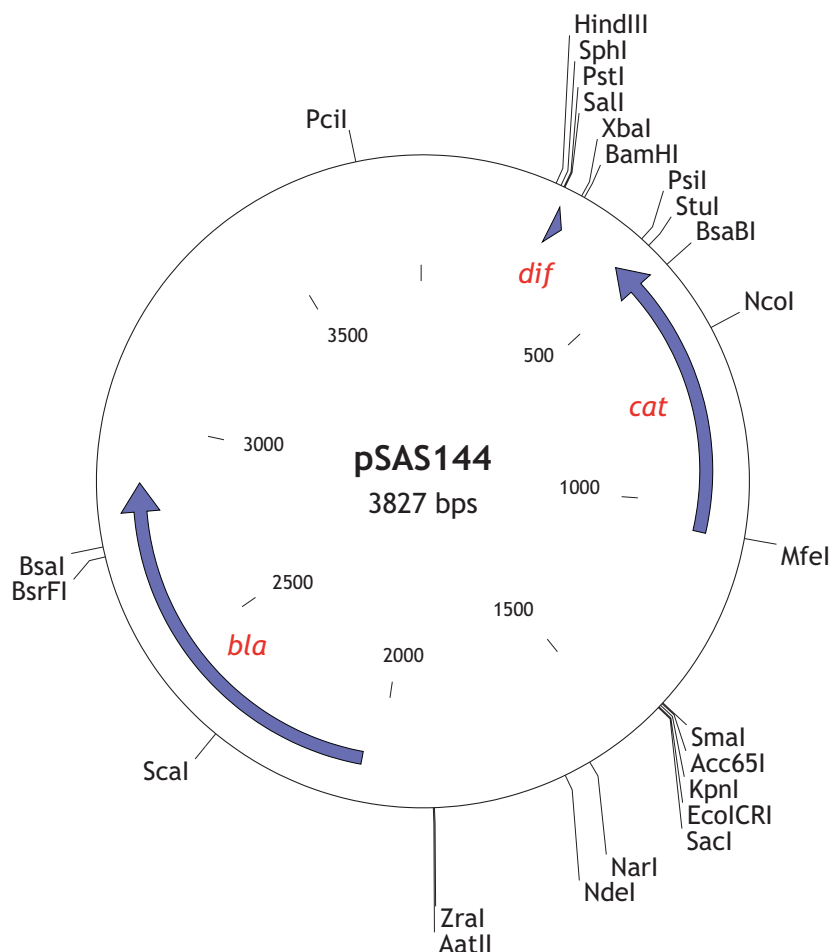


Bacillus Genetic Stock Center

New Product Announcement



BGSC Accession: ECE142

Original Code: DH5 α (pSAS144)

Reference: Sciochetti, S. A., P. J. Piggot, and G. W. Blakely. 2001. Identification and Characterization of the *dif* Site from *Bacillus subtilis*. *J. Bacteriol.* 183:1058-1068

Sequence: Not in database; available from BGSC at <http://www.bgsc.org/sequences/pSAS144.htm>

Features:

<i>dif</i>	<i>Bacillus subtilis</i> site-specific recombination target for resolving chromosome dimers
<i>cat</i>	encodes chloramphenicol acetyl transferase; selectable in either <i>E. coli</i> or <i>B. subtilis</i> (chloramphenicol 5 μ g/ml)
<i>bla</i>	encodes β -lactamase; selectable in <i>E. coli</i> only (ampicillin 100 μ g/ml)

Description: Integration vector; integrates by site-specific recombination between plasmid and chromosomal *dif* sequences via the host RipX-CodV system.

Construction: A 28 bp synthetic *dif* site was inserted between the pUC19 SalI and XbaI sites. A pDH32 NaeI-SmaI digestion fragment, containing *cat*, was inserted into the SmaI site of the resulting plasmid to produce pSAS144.

Use: pSAS144 integrates into *Bacillus subtilis* 168 at 166° of the chromosome, between bp 1941798 and 1941825 of the genomic sequence. Integration is *recA*-independent, instead making use of the host's system for resolving chromosome dimers via site-specific recombinases. Users simply insert a fragment of interest into one of the many available restriction sites. Transformation of competent *B. subtilis* with selection for chloramphenicol resistance recovers integrants.

Recipient strains: Any RipX+ CodV+ *B. subtilis* 168 derivative--even *recA* mutants--will serve as a recipient. It is not known if other Gram-positive bacteria may substitute, although it would be interesting to try.

Protocols: *B. subtilis* competent cell preparation and transformation